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# Herpes Simplex Virus (HSV)-Specific Human T-Cell Clones Recognize HSV Glycoprotein D Expressed by a Recombinant Vaccinia Virus

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**Human cytotoxic T-cell (CTL) clones that lyse autologous cells infected with herpes simplex virus (HSV) type 1 or 2 were generated by stimulating lymphocytes with a recombinant vaccinia virus (recombinant vaccinia–gD-1 virus) that expresses HSV type 1 glycoprotein D (gD-1). Furthermore, CTL clones generated with HSV type 1 or with cloned gD-1 lysed autologous cells infected with the recombinant vaccinia–gD-1 virus. Our findings thus showed that gD serves as a target antigen for human CTLs and that a recombinant vaccinia-gD virus activates HSV-specific human CTL.**

Herpes simplex virus (HSV) types 1 and 2 (HSV-1 and HSV-2, respectively) cause latent ganglionic infections in humans that can persist for life and result in recurrent oral and genital infections despite the usual presence of virus-neutralizing antibodies (6, 16). Studies with experimental animals have indicated that T-cell-mediated immunity, in addition to humoral immunity, plays an important role in resistance to HSV infections; adoptive transfer of HSV-immune T cells, including cytotoxic T cells (CTL), can clear infectious virus and contribute to the recovery of HSV-infected mice (9–11, 15, 17, 18, 21). At present, there is little information concerning which HSV antigens elicit T-cell-mediated immunity in humans and serve as target antigens for human CTL. HSV encodes approximately six major glycoproteins which are expressed on the surfaces of the virus and virus-infected cells (23). One of these, glycoprotein D of HSV-1 (gD-1), has been cloned and expressed by transfected or recombinant vaccinia virus-infected mammalian cells (7, 12, 13, 19). Immunization of experimental animals with purified gD-1 or with live recombinant vaccinia virus that expresses gD-1 (recombinant vaccinia–gD-1 virus) was found to protect against HSV-1 infections (2, 5, 7, 8, 13, 19). We report here that many human CTL clones generated with HSV-1 and all the CTL clones generated with purified gD-1 lysed autologous cells infected with the recombinant vaccinia–gD-1 virus, thus demonstrating that gD serves as a target antigen for HSV-specific human CTL. Furthermore, HSV-specific CTL clones were generated by stimulating human lymphocytes with a recombinant vaccinia-gD virus.

The recombinant vaccinia virus used for this study, vgD52, was constructed by using the WR strain of vaccinia virus and contains the entire coding sequence of the gD-1 gene of HSV-1 (strain KOS) (7). It induces the synthesis of an immunologically reactive glycosylated polypeptide that comigrates with authentic gD-1, and it induces serum neutralizing antibodies in mice. Furthermore, this recombinant was found to protect mice against a potentially lethal challenge with live HSV-1 or HSV-2, and a single vaccination also prevented the development of latent infections in the majority of the vaccinated animals (7).

To determine whether gD serves as a target antigen for HSV-specific human CTL, we tested the ability of HSV-1-directed CTL clones to lyse target cells infected with the recombinant vaccinia–gD-1 virus. We previously showed that human CTL clones isolated from HSV-1-stimulated peripheral blood lymphocytes (PBL) lysed either HSV-1- or both HSV-1- and HSV-2-infected autologous target cells and target cells sharing certain human leukocyte antigen (HLA) class II antigens with the clones (26, 27). The CTL clones used for this study were generated as previously described (26–28). Briefly, $10^5$ PBL from HSV-1-seropositive individuals were stimulated in microwells twice at weekly intervals either with purified gD-1 (1 μg/ml) that was cloned and expressed in mammalian cells (13) or with UV-light-inactivated HSV-1 ($10^5$ PFU/ml prior to UV light inactivation) in RPMI 1640 medium containing 10% heat-inactivated normal human serum (complete medium). Three days later, the stimulated cells were cloned by seeding single cells in microwells containing $5 \times 10^4$ X-irradiated (2,500 R) autologous PBL as antigen-presenting cells and UV light-inactivated HSV-1 in complete medium containing 5% lectin-free T-cell growth factor (TCGF; Cellular Products, Buffalo, N.Y.). The clones were expanded in TCGF and fed every 10 days with X-irradiated autologous PBL and UV light-inactivated HSV-1.

The results in Table 1 show examples of CTL clones that were generated by stimulation of PBL with purified, cloned gD-1 and that lysed $^{51}$Cr-labeled autologous target cells infected with HSV-1 or with the recombinant vaccinia–gD-1 virus but not with parental vaccinia virus. A total of 33 CTL clones were isolated from donor 2 PBL stimulated with gD-1, and all 33 lysed the recombinant vaccinia–gD-1 virus-infected cells. In contrast, HSV-1-specific CTL clones that were generated by stimulation with HSV-1 glycoprotein B-1 (30) failed to lyse target cells infected with the recombinant vaccinia–gD-1 virus (data not shown). Table 1 also shows several examples of CTL clones that were generated by stimulation of PBL with HSV-1 and that lysed autologous target cells infected with the recombinant vaccinia–gD-1

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TABLE 1. Lysis of autologous cells infected with the recombinant vaccinia–gD-1 virus by CTL clones generated with purified gD-1 or with HSV-1[a]

| Clone | % Specific $^{51}Cr$ release from autologous target cells infected with: | | |
|---|---|---|---|
| | HSV-1 | Recombinant vaccinia virus | Parental vaccinia virus |
| gD-1 stimulated | | | |
| Donor 1 | | | |
| 6-3 | 84.0 | 95.5 | 0.2 |
| 6-4 | 76.2 | 86.0 | 2.1 |
| 6-10 | 64.3 | 43.2 | −2.8 |
| Donor 2 | | | |
| 3-6 | 52.0 | 52.5 | 4.8 |
| 3-39 | 55.1 | 59.5 | −0.2 |
| 3-61 | 41.1 | 55.8 | 1.6 |
| 3-22 | 64.6 | 48.6 | −1.4 |
| HSV-1 stimulated | | | |
| Donor 1 | | | |
| 1-17 | 37.6 | 30.4 | 2.2 |
| 2-29 | 27.4 | 61.2 | −2.2 |
| 2-9 | 43.9 | 4.7 | 1.5 |
| Donor 3 | | | |
| 23 | 99.3 | 89.6 | −2.0 |
| 43 | 100.0 | 92.3 | −2.2 |
| Donor 2 | | | |
| 1-7 | 32.5 | 39.1 | −0.8 |
| 1-17 | 49.4 | 48.8 | 2.0 |
| 1-5 | 33.4 | 2.4 | 1.6 |

[a] CTL clones generated by stimulation of PBL from HSV-1-seropositive individuals 1, 3, and 2 with UV light-inactivated HSV-1 or with purified, cloned gD-1 were tested for their ability to lyse $^{51}Cr$-labeled autologous lymphoblastoid cell lines infected for 6 h with HSV-1, with parental WR strain vaccinia virus, or with the recombinant vaccinia–gD-1 virus, vgD52, at a multiplicity of infection of 10 PFU per cell in 6-h $^{51}Cr$ release assays. Effector cell/target cell ratios were 20:1. The percent specific $^{51}Cr$ release was calculated as follows: experimental release − spontaneous release (in medium alone) divided by maximal release (in detergent) − spontaneous release. The spontaneous $^{51}Cr$ release from the target cells did not exceed 15% of the maximum $^{51}Cr$ release. Values shown are the means from four replicate wells. The standard deviations did not exceed 12% of the means. Subclones isolated by recloning showed the same specificities as the parental clones, and all clones examined were at least 98% $T3^+$, $T4^+$ and 0% $T8^+$, as determined by immunofluorescence with monoclonal antibodies. Clones from donors 3 and 1, who shared no HLA class I or II antigens, did not lyse each other's virus-infected target cells.

virus. Approximately 70 and 50% of all the CTL clones we generated from HSV-1-stimulated donor 2 and 1 PBL, respectively, lysed recombinant vaccinia–gD-1 virus- but not parental vaccinia virus-infected autologous cells.

A number of different approaches have been used to study the target specificity of virus-directed CTL in inbred strains of mice. These have included the use of target cells that were (i) infected with virus and treated with inhibitors of glycosylation, (ii) transfected with DNA encoding particular viral antigens, (iii) infected with intertypic recombinant viruses, and (iv) infected with recombinant vaccinia viruses expressing genes for antigens of other viruses (1, 3, 4, 14, 24, 25, 28, 29). The ability to rapidly infect cells of many species of animals so that a majority express high levels of a desired antigen within a few hours has made the use of recombinant vaccinia viruses attractive. Moreover, the same recombinant virus can be used to infect cells with different major histocompatibility complex genes. The latter feature is an important advantage when studying the CTL specificity of outbred animals such as humans in which separate target cells must be made for each individual. Recent studies have indicated that cells infected with a recombinant vaccinia virus that expresses an influenza virus gene can serve as target cells for influenza virus-directed human CTL (14a). Our study demonstrates the usefulness of a recombinant vaccinia virus for elucidating the antigenic specificity of human CTL directed against HSV.

Experiments were carried out to determine whether HSV-specific human CTL can be generated by stimulation with the recombinant vaccinia-gD virus. PBL from HSV-seropositive individuals were stimulated with purified, UV light-inactivated recombinant vaccinia–gD-1 virus ($10^5$ PFU/ml prior to UV light inactivation) that fails to produce plaques but induces the expression of gD, as determined by immunofluorescence with a monoclonal antibody to gD-1. In our initial experiments, PBL stimulated twice with UV light-inactivated recombinant virus were cloned and maintained with UV light-inactivated recombinant vaccinia–gD-1 virus, X-irradiated autologous PBL as antigen-presenting cells, and TCGF. However, of 11 CTL clones isolated in this way, 6 lysed vaccinia virus-infected cells, presumably owing to the presence of vaccinia virus-specific memory T cells in individuals previously vaccinated against smallpox with vaccinia virus. Therefore, to select for the growth of HSV-specific CTL clones following two stimulations of PBL with

TABLE 2. HSV-1 type-specific and HSV-1 and HSV-2 type-common CTL clones generated with the recombinant vaccinia–gD-1 virus[a]

| Clone | % Specific $^{51}Cr$ release from autologous target cells infected with: | | | |
|---|---|---|---|---|
| | HSV-1 | HSV-2 | Recombinant vaccinia virus | Parental vaccinia virus |
| Type common | | | | |
| Donor 2 | | | | |
| 4-17 | 63.4 | 34.5 | 57.7 | −0.9 |
| 4-23 | 63.5 | 25.3 | 40.4 | 4.4 |
| 4-27 | 57.0 | 41.4 | 38.6 | 5.5 |
| 4-36 | 86.2 | 42.3 | 73.7 | 0.0 |
| 4-37 | 58.4 | 32.8 | 53.4 | 4.8 |
| Type specific | | | | |
| Donor 4 | | | | |
| 1-21 | 38.7 | 2.1 | 49.8 | 1.1 |
| 1-26 | 61.7 | 2.1 | 71.2 | 1.9 |
| Donor 2 | | | | |
| 4-3 | 40.7 | −4.4 | 25.3 | −0.9 |
| 4-7 | 74.8 | −0.7 | 54.3 | 2.4 |
| 4-13 | 63.7 | −2.6 | 47.7 | −0.1 |

[a] CTL clones were generated by stimulating PBL from HSV-seropositive individuals 2 and 4 twice at weekly intervals with purified, UV light-inactivated recombinant vaccinia–gD-1 virus prior to seeding single wells in microwells. The clones were expanded in TCGF-containing medium and fed each 7 to 10 days with X-irradiated autologous PBL and UV light-inactivated HSV-1. The cloned cells were tested for their ability to lyse autologous infected target cells in 6-h $^{51}Cr$ release assays at a ratio of 20 effector cells to 1 target cell as described in Table 1, footnote *a*. That the target cells which were infected with parental vaccinia virus and which were not lysed by the HSV-directed CTL clones were, however, susceptible to lysis was demonstrated by the findings that two clones generated by stimulation with the recombinant vaccinia virus lysed autologous target cells infected with either parental vaccinia virus or recombinant vaccinia virus but not with HSV (data not shown); these clones were considered to be directed against vaccinia virus.

the recombinant virus, we cloned the cells and maintained them in the presence of UV light-inactivated HSV-1. Table 2 shows several examples of such CTL clones that lysed HSV- and recombinant virus-infected cells but not parental vaccinia virus-infected cells. Of 27 CTL clones generated from donor 2, 25 lysed HSV-1- and recombinant virus-infected cells but not parental vaccinia virus-infected cells; of these 25, 10 also lysed autologous HSV-2-infected cells. The isolation of CTL clones that apparently recognized type-common epitopes of gD on HSV-1 and HSV-2 is consistent with the high degree of homology between the genes for gD-1 and HSV-2 glycoprotein D (gD-2) (12) and the broad serological cross-reactivity of gD-1 and gD-2 (20). None of these clones lysed allogeneic HSV-1- or recombinant virus-infected cells that share no HLA class I or II antigens with the clones. Consistent with our earlier finding that HSV-1-stimulated clones are react with monoclonal antibodies to T-cell antigens T3 and T4 (26, 27), the clones generated with the recombinant virus were also approximately 98% $T3^+$, $T4^+$ and 0% $T8^+$. Subclones isolated from the clones exhibited the same phenotype and pattern of specificity as did the parental clones.

In addition to our finding that gD serves as a major target antigen for HSV-specific CTL clones, this study also shows that a recombinant vaccinia-gD can activate HSV-specific CTL from humans who have been naturally exposed to HSV. The determination of whether such a recombinant virus will prime HSV-specific human CTL in vivo would require human vaccine trials. Recent findings that a recombinant vaccinia virus expressing an influenza virus protein can successfully vaccinate against influenza virus infections (22) and can prime CTL against influenza virus in experimental animals (1) support the contention that a recombinant vaccinia-gD virus will prime HSV-specific CTL in humans.

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